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Journal of Chromatography B, 753 (2001) 401–408

JOURNAL OF CHROMATOGRAPHY B

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Liquid chromatographic–mass spectrometric determination of celecoxib in plasma using single-ion monitoring and its use in clinical pharmacokinetics

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Received 8 September 2000; received in revised form 11 October 2000; accepted 2 November 2000

Abstract

Celecoxib is a cyclooxygenase-2 specific inhibitor, that has been recently and intensively prescribed as an anti-inflammatory drug in rheumatic osteoarthiritis. A robust, highly reliable and reproducible liquid chromatographic–mass spectrometric assay is developed for the determination of celecoxib in human plasma using sulindac as an internal standard. The run cycle-time is <4 min. The assay method involved extraction of the analytes from plasma samples at pH 5 with ethyl acetate and evaporation of the organic layer. The reconstituted solution of the residue was injected onto a Shim Pack GLC-CN, C_{18} column and chromatographed with a mobile phase comprised of acetonitrile–1% acetic acid solution (4:1) at a flow-rate of 1 ml/min. The mass spectrometer (LCQ Finnigan Mat) was programmed in the positive single-ion monitoring mode to permit the detection and quantitation of the molecular ions of celecoxib and sulindac at m/z 382 and 357, respectively. The peak area ratio of celecoxib/sulindac and concentration are linear (r^2>0.994) over the concentration range 50–1000 ng/ml with a lowest detection limit of 20 ng/ml of celecoxib. Within- and between-day precision are within 1.58–4.0% relative standard deviation and the accuracy is 99.4–107.3% deviation of the nominal concentrations. The relative recoveries of celecoxib from human plasma ranged from 102.4 to 103.3% indicating the suitability of the method for the extraction of celecoxib and I.S. from plasma samples. The validated LC–MS method has been utilized to establish various pharmacokinetic parameters of celecoxib following a single oral dose administration of celecoxib capsules in two selected volunteers. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Celecoxib

1. Introduction

Celecoxib (Celebrex) is a cyclooxygenase (COX) inhibitor that exhibits in vitro and in vivo selectivity for COX-2 over COX-1. Celecoxib possesses anti-inflammatory, analgesic and antipyretic activities. The drug has similar efficacy as conventional non-steroidal anti-inflammatory drugs (NSAIDs) in improving the symptoms of osteoarthritis and rheumatoid arthritis, but it is associated with a lower incidence of gastrointestinal ulceration and complications [1]. This promising gastrointestinal safety profile, together with sustained symptomatic pain relief, places celecoxib as an alternative to the conventional NSAIDs in the treatment of rheumatoid

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PII: S0378-4347(00)00574-0

diseases, particularly in patients at high risk of developing gastrointestinal problems. The chemopreventive effect of celecoxib on colon cancer [2] and its clinical effects on blood platelets [3] renal [4,5], hepatic and pancreatic [6] functions have been recently investigated and reported. The metabolism and excretion patterns of radiolabeled [^{14}C]celecoxib were determined in healthy male volunteers. Clinical studies indicated that celecoxib is metabolized in the liver via the oxidative pathway to the corresponding alcohol and carboxylic acid and is removed from the body by renal excretion as a glucuronide metabolite [7]. The clinical pharmacokinetics and pharmacodynamics of celecoxib in humans have been recently reported [8]. For clinical investigations such as pharmacokinetic studies, development of sensitive and specific analytical techniques for the determination of celecoxib in biological samples is highly required. As reported in the literature, only one liquid chromatographic (LC) assay for the determination of celecoxib in pharmaceutical dosage forms was described [9]. However, no method for the determination of celecoxib in biological matrices was reported. Although, LC–mass spectrometry (MS) was shown to be sophisticated and expensive, however, the technique was proven to be rugged for the detection and quantitation of drugs at very low concentrations. LC–MS and LC–MS–MS analyses applying ion-trap technology (Finnigan Mat) were used for the detection of drugs in the presence of impurities or metabolites as well as for the quantitation of drugs in complex matrices [10–12]. This work reports on the application of atmospheric pressure ionization liquid chromatography–mass spectrometry (API-LC–MS) using single-ion monitoring (SIM) for the quantitation of celecoxib in human plasma. Data supporting the linearity, limits of detection, precision and accuracy are presented. The utility of the developed method in a preliminary clinical study to determine the pharmacokinetic parameters of celecoxib following oral administration of the drug in selected subjects is demonstrated.

2. Experimental

2.1. Materials

Pharmaceutical-grade celecoxib and sulindac powder were used. Human plasma samples were donated by the Central Blood Bank, Kuwait. HPLC-grade solvents of acetonitrile and ethyl acetate were used for mobile phase preparation and liquid–liquid extraction. Water was purified by a Milli-Q system from Millipore. Other chemicals and reagents used were of analytical grade.

2.2. Instrumentation

2.2.1. Liquid chromatography

An LC pump (Spectra System P 2000) and an AS 3000 autosampler were used. These components were directly controlled by an LCQ data system. The mobile phase consisted of acetonitrile–1% acetic acid solution (4:1, v/v) and was pumped at a flow-rate of 1 ml/min. The column was a Shim Pack GLC-CN C_{18}, 5 μm, 150 mm×6 mm and was operated at ambient temperature (~22°C).

2.2.2. Mass spectrometry

The high-performance liquid chromatography (HPLC) analyses were performed using an LCQ mass spectrometer (Finnigan Mat, USA) with an API source. The atmospheric pressure chemical ionization (APCI) probe was used with the following parameters; vaporization temperature 450°C, sheath gas flow-rate 80 units, discharge current 8 μA, capillary temperature 150°C, capillary voltage 32 V and tube lens offset 55 V, scanning mode was in the positive SIM mode at m/z 382 and 357 for celecoxib and sulindac, respectively. Analytical data were acquired by LCQ software.

2.3. Standard solutions

Stock solutions of celecoxib and the internal standard (sulindac) were prepared in acetonitrile at concentrations 1 μg/μl and were stored at 4°C. Working standards at a concentration of 10 ng/μl were daily prepared in acetonitrile for preparation of the calibration curves.

2.4. Assay procedure

A 1-ml aliquot of the collected plasma sample from a human volunteer was pipetted into a 15-ml screw-capped glass tube. A 100-μl aliquot of the internal standard solution (10 ng/μl) was added and

properly mixed with plasma. The contents of the tube were mixed with a 1-ml volume of 0.1 *M* acetate buffer solution (pH 5) and were extracted with 7 ml ethyl acetate on an orbital shaker for ~10 min at a moderate shaking rate. After centrifugation of the sample at 1500 *g* for 10 min, the organic layer was removed, transferred to a 10-ml centrifuge tube and evaporated to dryness under stream of nitrogen gas at 50°C. The residue was reconstituted in ~100–200 μl of the mobile phase and a 20-μl aliquot was injected into the system. The concentration of celecoxib in the sample was determined from the least-squares regression equation of a calibration curve constructed by plotting the peak area ratio of celecoxib/sulindac (I.S.) versus celecoxib standards. A standard plot was established by spiking 1-ml blank plasma samples with 5–100-μl aliquots of celecoxib (10 ng/μl) and 100-μl aliquot of I.S. (10 ng/μl) in acetonitrile. The plot will cover the concentration range 50–1000 ng/ml of celecoxib in plasma.

2.5. Limit of quantitation (LOQ) and limit of detection (LOD)

Control plasma samples spiked with celecoxib at concentrations 50, 30 and 20 ng/ml ($n=5$) were prepared and analyzed as previously described. The precision expressed as relative standard deviation (RSD) and accuracy expressed as % deviation from the nominal concentrations at the LOQ were determined for each concentration.

2.6. Preliminary clinical study

Two healthy male volunteers of comparable age (~35 years) and weight (~70 kg) participated in this study. The volunteers were asked to stop taking any medicines at least 1 week before the study. After overnight fasting, each volunteer received an oral dose of 200 mg of celecoxib (two capsules, each containing 100 mg of celecoxib). The volunteers continued fasting for at least 3 h after capsule intake. Blood samples were drawn into heparinized tubes before drug administration (blank) and after drug intake for 24 h. The blood samples were centrifuged at 1500 *g* for 10 min and the plasma fractions were rapidly separated and frozen at −20°C pending analysis. The plasma concentrations were utilized to calculate the pharmacokinetic parameters for each volunteer using PK Solutions 2.0, Noncompartmental Pharmacokinetics Data Analysis (Summit, Research Services, UK) software.

3. Results and discussion

3.1. Method development

Celecoxib is a newly prescribed COX-2 specific inhibitor, whose therapeutic plasma levels are <1 μg/ml. Conventional HPLC methods using either UV or photodiode array detection were found to be insensitive and non-specific. Severe peak overlapping of plasma constituents with the target peaks of celecoxib and sulindac (I.S.) was observed. Furthermore, the measured drug concentrations were far from the expected plasma concentration range of celecoxib to be monitored for pharmacokinetic studies. The use of LC–MS permits elimination of the background interference arising from the complex matrices and allows detection of celecoxib at levels within the expected therapeutic concentration ranges.

Preliminary investigations have shown that celecoxib and sulindac (I.S.) are good candidates for LC–MS analysis, as both compounds were detected and quantified at considerably low concentrations. Although celecoxib and sulindac were not completely resolved, however, their respective chromatograms were perfectly extracted from the total ion chromatogram (TIC) at m/z 382 and 357, respectively. The high percentage of acetonitrile in the mobile phase ensured a rapid and complete elution of compounds from the column. The acidity of the mobile phase permits better detection of the analytes as positive molecular ions. Extraction of celecoxib and I.S. from plasma at different pH values was examined using various non-polar solvents. Ethyl acetate was found to be most suitable for extracting the investigated compounds from plasma at pH 5. Under these conditions, no interference of plasma matrix was observed in the chromatograms of celecoxib and I.S. when the samples were measured using MS in the SIM mode. This might prove the high specificity of LC–MS compared to HPLC for the analysis of celecoxib in plasma. The MS conditions were optimized and an MS setting program was designed.

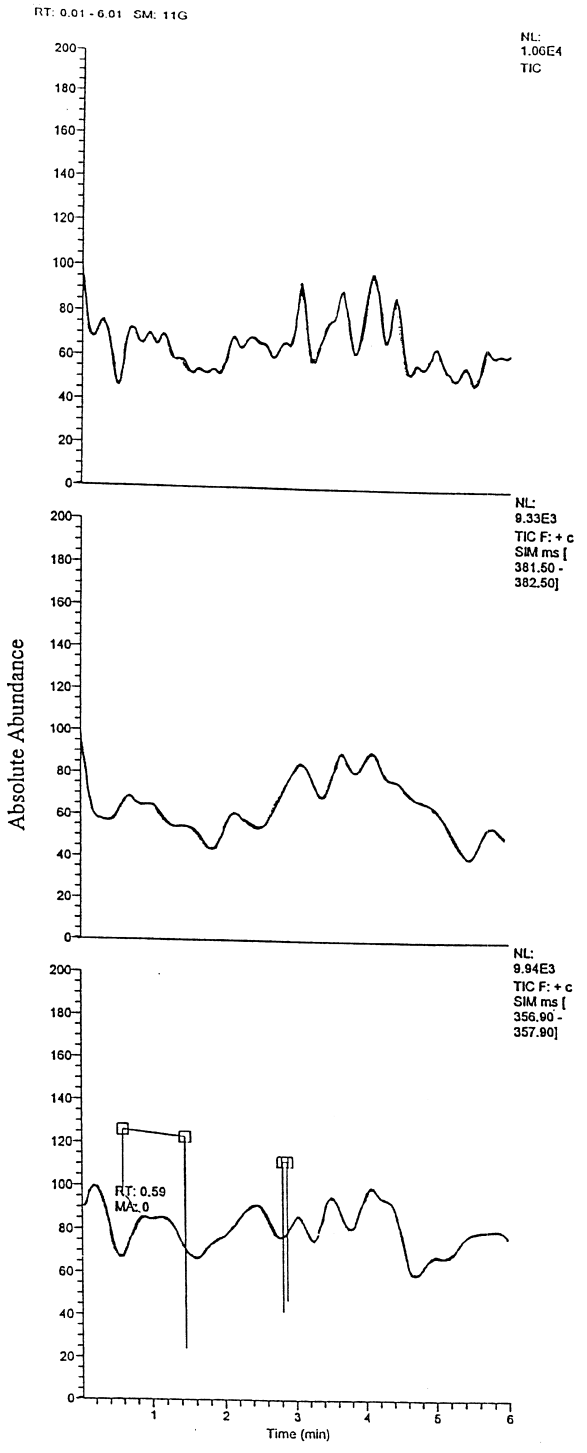

Fig. 1. Representative LC–MS chromatograms from blank human plasma monitored at m/z 382 and 357, respectively.

APCI as an ionization source with a vaporization temperature of 450°C was selected. SIM programs at 382.0±1 (celecoxib) and 357.0±1 (I.S.) were designed for specific detection and quantitation of celecoxib and I.S. in plasma. Typical ion chromatograms of blank plasma, spiked plasma with known concentration of celecoxib and collected plasma samples from volunteers are displayed in Figs. 1–3, respectively. As shown, celecoxib and I.S. appear at ~3.47 and 3.56 min, respectively. The reproducibility of retention time measurements ranges from 0.14 to 1.19% RSD.

3.2. Validation of LC–MS assay

3.2.1. Linearity

The quantitation of celecoxib in plasma samples was carried out by determining the slope (b), intercept (a) and regression coefficient (r) of the calibration curves of the peak area ratio of celecoxib/sulindac versus celecoxib concentration. Using linear regression analysis, the data confirmed linear relationships over the selected concentration range. The calibration curves were typically described by the least-square equation: $y=0.0274(\pm0.0017)+0.0015(\pm0.0003)x$ ($n=8$), where y corresponds to the peak area ratio of celecoxib to the I.S. and x refers to the concentration of celecoxib added to plasma over a concentration range 50–1000 ng/ml. Standard curves of celecoxib were constructed on 5 different days to determine the variability of slopes and intercepts. The results showed little day-to-day variability and gave acceptable linearity (r^2: 0.9936±0.003) over the plasma concentration range studied. Furthermore, the linearity data confirmed satisfactory stability of celecoxib in the spiked plasma samples at room temperature.

3.2.2. Limits of quantitation and detection

The LOQ and LOD of celecoxib in plasma were determined by analyzing plasma samples spiked with celecoxib at relatively low concentrations (20–50 ng/ml) using the developed LC–MS method under the described conditions. The LOQ for celecoxib in plasma was found to be 50 ng/ml (mean predicted concentration 41.0±4.2 ng/ml). This concentration yielded an RSD of 10.2% and an accuracy of −9.0% expressed as (% deviation) of the nominal con-

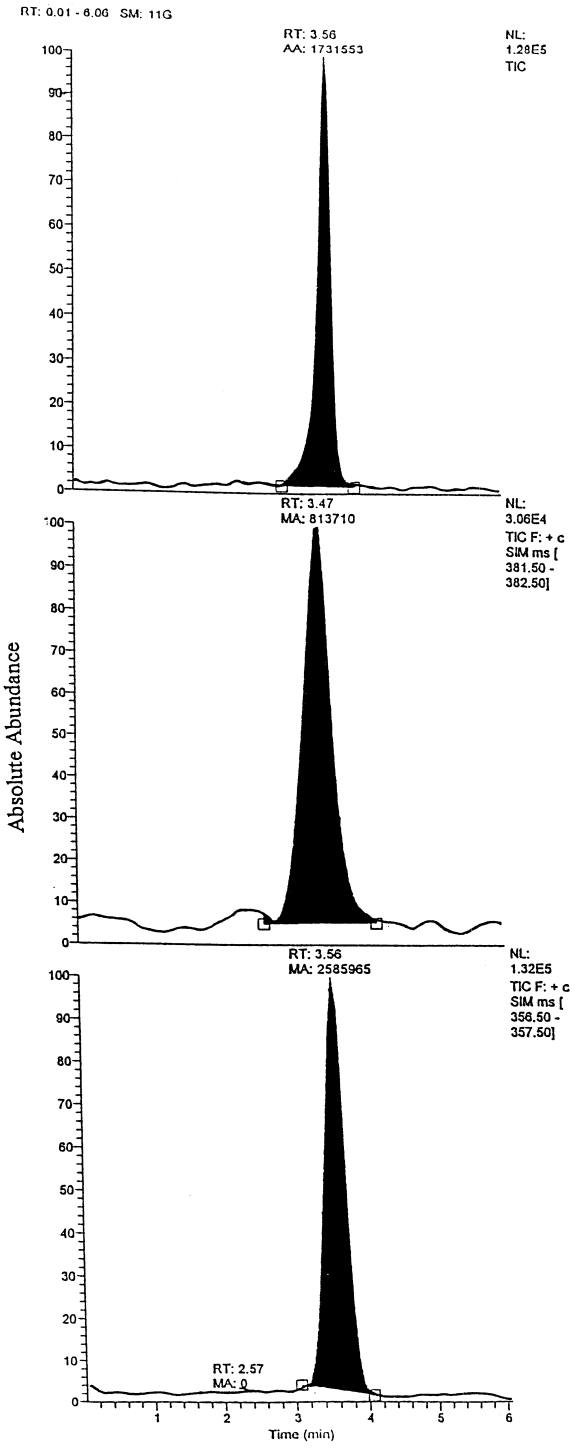

Fig. 2. Representative LC–MS chromatogram from extracted human plasma spiked with 200 ng/ml celecoxib, 1000 ng/ml I.S. and monitored at m/z 382 and 357, respectively.

centration. On the other hand, the LOD for celecoxib in plasma was 20 ng/ml.

3.2.3. Specificity

Blank human plasma samples from three different sources were collected and inspected for the presence of interfering peaks at the retention times of celecoxib and I.S. measured at the above molecular mass ions. In addition, conventional NSAIDs which may be co-administered with celecoxib such as diclofenac, naproxen, ibuprofen, and indomethacin were tested for their possible interference with LC–MS analysis of celecoxib. No interference was detected which confirmed the high specificity of the developed LC–MS for the determination of celecoxib in plasma.

3.2.4. Precision and accuracy

To evaluate the precision and accuracy of LC–MS method, drug-free plasma samples were spiked with different concentrations of celecoxib at concentrations within the expected drug plasma levels to be monitored for pharmacokinetic studies. The spiked samples were analyzed on the same day (within-day) and on different days (between-day) to determine the assay variability. The samples were frozen at −20°C and measured for 10 days. Mean values of the RSD and % deviation ($n=10$) are shown in Tables 1 and 2. The data revealed good precision and accuracy of LC–MS as indicated by the relatively low values of RSD (<5%) and % deviation (<10%). The results also indicated that the frozen plasma samples containing celecoxib are stable for at least 10 days.

3.2.5. Extraction recovery

Control plasma samples containing celecoxib at concentrations 250, 500 and 750 ng/ml and I.S. at concentrations 1000 ng/ml were extracted and analyzed using LC–MS as previously described. The drug concentrations were determined from a calibration curve relating the peak area ratio of celecoxib/I.S. and concentration. The results (Table 3) indicated satisfactory relative recovery percentages of celecoxib ($n=5$) at the specified concentration levels and confirmed the suitability of the method for the extraction of celecoxib and I.S. from plasma samples under the described conditions.

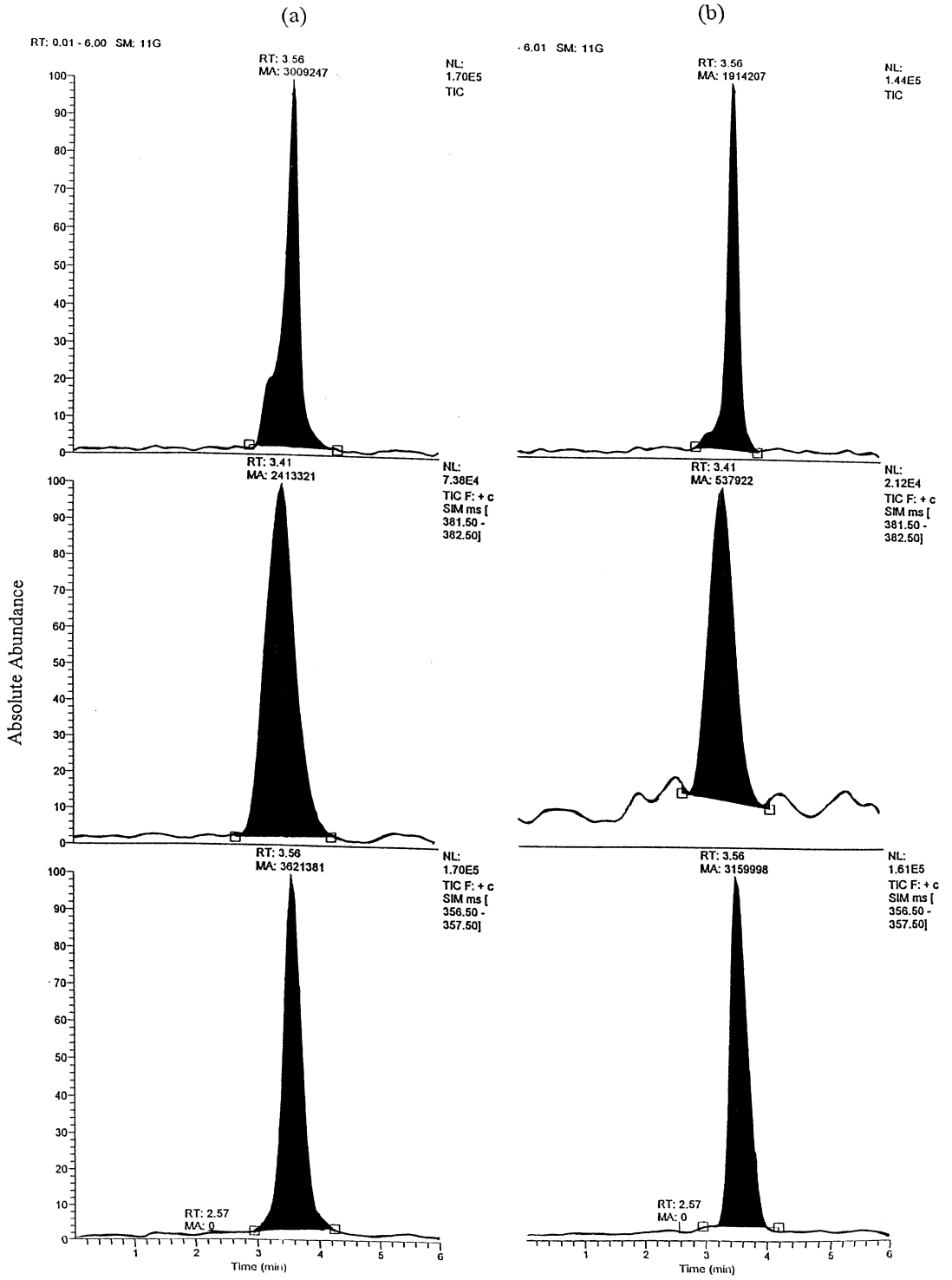

Fig. 3. Representative LC–MS chromatograms from extracted human plasma samples collected 1 h (a) and 24 h (b) after dosing with celecoxib capsules (200 mg) to a healthy male volunteer.

Table 1
Within-day precision and accuracy data for LC–MS analysis of celecoxib in plasma

Standard concentration		Precision[a] (%)	Accuracy[b] (%)
Nominal (ng/ml)	Mean (n=10) analyzed		
200	199.4	2.56	99.7
600	596.4	2.44	99.4
800	811.5	1.58	101.4

[a] Expressed as the relative standard deviation (RSD).
[b] Expressed as [mean % deviation=(mean calculated concentration/nominal concentration×100].

Table 2
Between-day precision and accuracy data for LC–MS analysis of celecoxib in plasma stored at −20°C

Standard concentration		Precision[a] (%)	Accuracy[b] (%)
Nominal (ng/ml)	Mean (n=10) analyzed		
200	214.5	4.00	107.3
1000	987.7	3.03	98.8

[a] Expressed as the relative standard deviation (RSD).
[b] Expressed as [mean % deviation=(mean calculated concentration/nominal concentration×100].

3.3. Pharmacokinetic studies

In a trial to prove the utility of the developed method in clinical studies, the LC–MS method was utilized to determine the pharmacokinetic parameters of celecoxib in humans following an oral administration of a single dose of celecoxib to two selected healthy male volunteers. Samples were collected at specified intervals and analyzed by LC–MS procedure. Representative plasma concentration–time profiles from two volunteers receiving 200-mg oral doses of celecoxib are shown in Fig. 4. The calculated pharmacokinetic parameters using, PK Solutions 2.0 software, are presented in Table 4. The data were comparable for both volunteers and were in good agreement with the reported data [8].

Table 3
Mean relative recovery percentages of celecoxib from human plasma (n=5)

Concentration spiked (ng/ml)	Mean (%)±SD
250	102.9±6.4
500	103.3±1.9
750	102.4±2.5

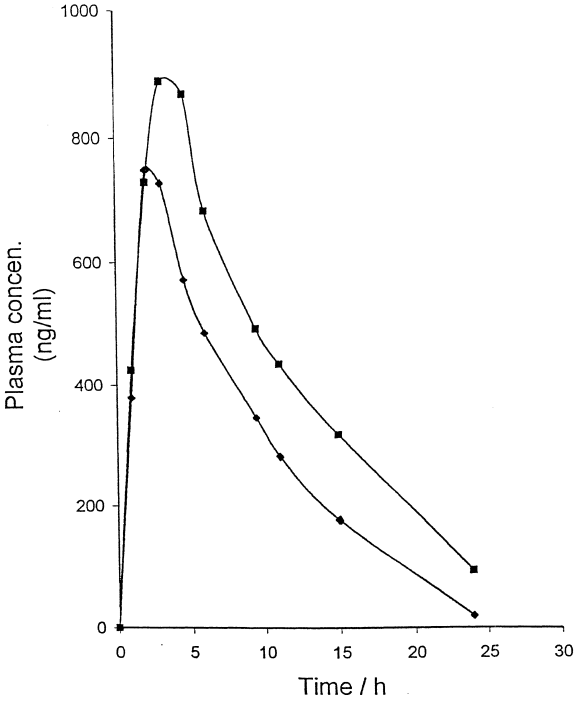

Fig. 4. Representative plasma concentration–time profiles of celecoxib after administration of oral dose (200 mg) of celecoxib to two male volunteers.

In conclusion, an LC–MS assay has been developed for the determination of celecoxib in human plasma using SIM. The method has been found to be fast and reliable. Due to lack of the analytical techniques for the determination of celecoxib in biological matrices, the developed LC–MS method is suitable for drug analysis in plasma samples

Table 4
Calculated pharmacokinetic parameters of celecoxib following oral administration of a 200-mg oral dose to two healthy subjects

Parameter[a]	Volunteer 1	Volunteer 2
C_{max} (ng/ml)	855.6	947.0
t_{max} (h)	2.5	3.5
AUC_{0-t} (ng h/ml)	6612.8	7963.3
$AUC_{0-\infty}$ (ng h/ml)	6676.2	7963.3
K_e (1/h)	0.093	0.071
$t_{1/2}$ (h)	7.44	9.79

[a] Parameters: C_{max}, peak plasma concentration; t_{max}, time of peak concentration; AUC, area under the curve; K_e, elimination rate constant; $t_{1/2}$, elimination half-life.

during clinical investigations such as pharmacokinetic studies.

Acknowledgements

The authors would like to thank the Kuwait University for the financial support of this work through the Research Grant FDC119. The authors are also indebted to Professor D. Biggs for his encouragement and support.

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